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mTOR inhibitors and risk of ovarian cysts: a systematic review and meta-analysis

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ABSTRACT

Objective To summarise the available evidence on frequency of ovarian cyst development during mammalian target of rapamycin inhibitors (mTORi) treatment.

Methods PubMed/Medline and EMBASE databases were searched, from 1990 up to March 2020, using the following keywords: 'tacrolimus', 'sirolimus', 'temsirolimus', 'everolimus', 'deforolimus', 'mTOR' and 'ovarian cysts' (Limit: Human, English, full article). Studies were selected for the review if they met the following criteria: clinical studies, studies reporting original data, studies reporting the number of patients using mTORi, studies reporting the number of patients with ovarian cysts.

We selected 7 of 20 retrieved studies. Study design, population, sample size, criteria for diagnosis of ovarian cysts, drug doses and follow-up length were extracted. Pooled estimate of incidence was calculated for ovarian cysts as a percentage, with 95% CI.

Results Four hundred-six women were included in the selected studies. The pooled incidence was 37.0% (95% CI 16.0% to 58.1%) for all ovarian cysts, and 17.3% (95% CI 5.6% to 29.1%) for clinically significant ovarian cysts. Based on two articles, comparing mTORi and non-mTORi for immunosuppression, pooled OR for ovarian cyst incidence was 4.62 (95% CI 2.58 to 8.28).

Conclusion Ovarian cyst development is a common adverse event during immunosuppression treatment with mTORi. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery.

Strengths and limitations of this study

- Due to the widespread role of mammalian target of rapamycin (mTOR), mTOR inhibitors (mTORi) may impact different organs and systems causing side effects that could be serious and/or debilitating.
- The mTOR signalling pathway is known to regulate ovarian function; thus it is conceivable that mTORi may affect ovarian activity.
- In the early 2000s, observational data have suggested that mTORi, sirolimus in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.
- This study summarises the available evidence on frequency of ovarian cyst development during mTORi treatment.
- Most studies included an extremely limited number of subjects and although meta-analyses provide an explicit method for synthesising evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study.

INTRODUCTION

The mammalian target of rapamycin (mTOR) kinase regulates cell growth and metabolism in response to intracellular and extracellular energetic stimuli and growth factors. The importance of mTOR in health and diseases has pushed the development of drugs that inhibit mTOR signalling (mTOR inhibitors, mTORi), including rapalogs, such as sirolimus (SRL), temsirolimus, tacrolimus (TAC), everolimus and deforolimus, which complex with FK506-binding protein 12 to inhibit mTOR complex 1 activity in an allosteric manner, or the more recent ATP-competitive mTORi (such as dactolisib), which targets the catalytic site of the enzyme.[1]

mTORi are used as targeted therapy for tumours (in particular renal carcinoma). Further mTORi inhibit T-cell proliferation and proliferative responses induced by several cytokines, including interleukin 1, interleukin 2, interleukin 3, interleukin 4, interleukin 6, insulin-like growth factor, platelet-derived growth factor and colony-stimulating factors and they have been used in combination therapy with corticosteroids and cyclosporine (CsA) in patients who received kidney transplantation to prevent organ rejection, and in the treatment of rheumatoid arthritis.[1]

Due to the widespread role of mTOR, mTORi may impact different organs and systems causing side effects that could be serious and/or debilitating. The mTOR signalling pathway is known to regulate

ovarian function[2]; thus, it is conceivable that mTORi may affect ovarian activity. Along this line, in the early 2000s, observational data have suggested that mTORi, SRL in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.

In this paper, we reviewed the available data on the reported frequency of ovarian cysts, during treatment with mTORi SRL.

METHODS

We searched the PubMed (National Library of Medicine, Washington, District of Columbia, USA) and EMBASE databases from 1990 up to March 2020 using different combinations of the following keywords: (a) 'tacrolimus', 'sirolimus', 'temsirolimus', 'everolimus', 'deforolimus' and 'mTOR' and 'ovarian cysts' (Limit: Human, English, full article) (see online supplemental file 1).

Furthermore, we reviewed reference lists of retrieved articles to search for other pertinent studies.

Two authors reviewed the papers and independently selected the articles eligible for the systematic review and extracted data. Any disagreements were submitted to a third reviewer to solve.

Inclusion criteria

Studies were selected for the review if they met all the following criteria: clinical studies, studies reporting original data, studies reporting number of patients using mTORi, studies reporting number of patients with ovarian cysts.

Exclusion criteria

Reviews, commentaries and case reports were excluded from the review.

The present review and meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guideline.[3]

Patients and public involvement

It was not appropriate to involve patients or the public in our research.

Data extraction

A Patient, Intervention, Comparator, Outcome, Study design structure was used to develop the study questions and the inclusion/exclusion criteria. The question was, 'Is there a relationship between mTORi sirolimus and ovarian cysts?' (table 1).

For each study, the following information was extracted: first author's last name; year of publication; country of origin; design of the study; number of subjects treated with SRL; age if present; criteria for the diagnosis of ovarian cysts; type and dose of drug; length of follow-up; number of women with newly diagnosed ovarian cyst. Further, we have collected information on the clinically significant ovarian cysts. This group includes symptomatic cysts, cyst >6 cm and cysts requiring surgery (see below).

Table 1 Patient, Intervention, Comparator, Outcome, Study criteria for inclusion and exclusion of studies

Parameter	Inclusion criteria	Data extraction
Patient	Women treated with mTOR inhibitors	Location, age, type of patients
Intervention	mTOR inhibitors	Dose and duration
Comparator	No treatment	Group definition
Outcome	Ovarian cysts yes/no	Number of cases, type of assessment
Study	Cross-sectional, cohort, case-control studies, clinical trials	Type of study design

mTOR, mammalian target of rapamycin.

Quality assessment

The quality of the studies included in the review was assessed using the Newcastle-Ottawa Scale (NOS).[4]

This instrument was developed to assess the quality of non-randomised studies, specifically cohort and case-control studies. Studies were judged based on three broad categories: selection of study groups, comparability of study groups and assessment of outcome (cohort studies) or ascertainment of exposure (case-control studies). The maximum score was 9.

Randomised controlled trials (RCTs) were evaluated using the revised Cochrane risk-of-bias tool for randomised trials.[5]

Data synthesis

The primary outcomes assessed were ovarian cyst (overall and clinically significant) in the total series and, if available, separately for premenopausal and postmenopausal women.

For each study with binary outcomes, we calculated the 95% CI of the estimated proportion. To evaluate the association between ovarian malignancy and menopausal status, we computed Pearson χ^2 test for heterogeneity and relative p value.

We used Metaprop, a command implemented in Stata to compute meta-analysis of proportions (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, Texas, USA: StataCorp LP). Freeman-Tukey method was applied to include, in the computation, the studies with outcome proportion equal zero.[6]

Estimates of proportion and 95% CI were calculated by using random effect model. To evaluate heterogeneity among studies, heterogeneity χ^2 p value was also reported. We assessed the heterogeneity among studies using the $\chi 2$ test[7] and quantified it using the I2 statistic. Results were defined as heterogeneous for p values less than 0.10. We computed summary estimates for ovarian cysts. We also rerun the analysis excluding the most extreme result, to evaluate if the summary estimate substantially changed.

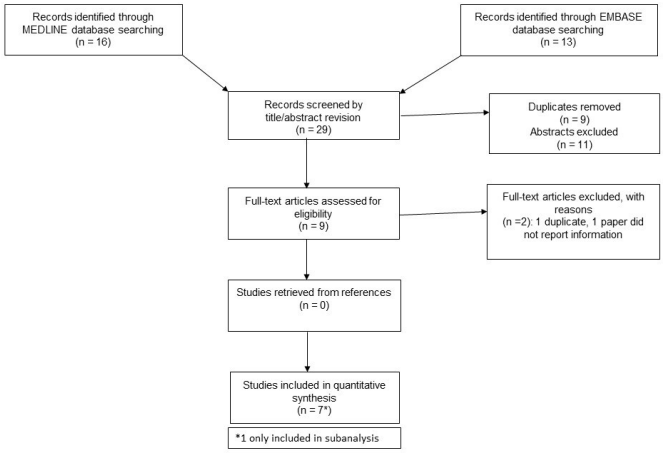

Figure 1 Flow chart of selected studies.

RESULTS

The initial search retrieved 16 abstracts from Pubmed, and 13 from EMBASE. Nine publications were retrieved both in Pubmed/Medline and EMBASE and 11 were excluded after reviewing abstracts: five laboratory studies, three case reports, one not including drugs of interest, and two reviews.

Thus, nine publications remained to be fully read.[8–16] One paper was excluded because it was duplicate[11] and another because the number of cases of ovarian cysts was not reported, although they were described as 'very frequent'.[16] One paper[13] reported the update of a previous one;[12] thus, the latter[12] was excluded from the main analysis but included in the subanalysis for menopausal status, since this information was missing in the updated report.[13]

figure 1 shows the flow diagram of the literature search results.

A total of seven studies have been identified: they were conducted in samples of women with type 1 diabetes mellitus (T1DM) who underwent allogeneic islet transplantation (AIT),[8 12 13] in women with polycystic kidney disease[10] and in renal transplant recipients.[9 14 15] Main methodological characteristics are presented in table 2.

Three studies were retrospective chart review,[8 9 15] two were cohort studies[12 13] and two were RCTs.[10 14] Three studies included women with T1DM who underwent allogenic islet transplantation,[8 12 13] three kidney transplantation recipients[9 14 15] and one study enrolled women with autosomal dominant polycystic kidney disease.[10]

Diagnosis of ovarian cysts was based on pelvic ultrasound examination in four studies[8 9 12 13] with MRI without contrast in one study,[10] whereas two did not report the diagnostic criteria.[14 15]

SRL was given at increasing dose to reach serum levels ranging from 7 to 15 ng/mL. In one study SRL was given at doses of 1.3–1.5 mg SRL per day.[11] TAC target was level 3–6 ng/ mL when given in association with SRL[12 13] and or 10 ng/mL when used in association with mycophenolate mofetil (1 g two times per day as tolerated).[8]

Overall, the considered studies included 406 women who received SRL alone or in combination with other drugs, with mean follow-up ranging from 12 to 95 months.

Quality of selected studies

Both Braun *et al* (10c) and Gaber *et al*[14] had low risk of bias according to the Cochrane risk of bias tool (table 3).

As regards observational cohorts, using the Newcastle-Ottawa Scale tool, study quality was deemed good (9 out of 9) in Bachmann *et al*'s paper.[9] Alfadhli *et al*'s study was of some concern because it was unclear if baseline ultrasound scans were detailed enough to identify ovarian cysts.[8] Del Olmo Garcia *et al*[13] and Ignjatović *et al*[15] presented mainly descriptive articles, including 18 (13 of whom already included in the paper by Cure *et al*[12]) and 6 women, respectively. Therefore, the possibility of some NOS quality item evaluation was debatable (ie, if sample size was too little to control for important factors or if a not exposed cohort did exist).

Main results

Table 4 reports the frequency of ovarian cysts in women treated with SRL, SRL+TAC and SRL or everolimus. Two studies[8 12] reported the frequency in strata of menopausal status, suggesting that premenopausal women were at higher risk of developing ovarian cysts during mTORi treatment.

Systematic review

Gaber *et al*[14] conducted a RCT to evaluate the efficacy and safety of SRL plus TAC versus SRL plus CsA in high-risk renal allograft recipients. A total of 202 women were randomly assigned before transplant to receive SRL–TAC (104 women) or SRL–CsA (98 women) with corticosteroids. Patients randomly assigned to SRL–TAC received a 10 mg loading dose of SRL on days 1 and 2, and 5 mg one time a day, thereafter, adjusted to achieve whole blood trough concentrations from 10 to 15 ng/mL (measured by high performance liquid chromatography methodology). Up to 0.2 mg/kg/day of TAC was administered in divided oral doses (two times per day) to achieve whole blood concentrations from 10 to 15 ng/mL between day 1 and week 2, from 5 to 10 ng/mL between weeks 2 and 26, and from 3 to 5 ng/mL between weeks 26 and 52 (measured by TDx monoclonal antibody assay or equivalent methodology). Patients randomly assigned to SRL–CsA received a larger 15 mg loading dose of SRL on day 1, and 5 mg one time a day, thereafter, adjusted to achieve the same whole blood trough concentrations as the patients assigned to SRL–TAC. One case of ovarian cyst was observed in the SRL–TAC group (1.0%) and seven in the SRL–CsA group (7.1%) (p=0.031). In this study, no information on severity of cysts (ie, for example dimension or presence of pain) was reported.

Alfadhli *et al*[8] conducted a chart review retrospective study in 57 women who underwent islet transplantation and received maintenance immunosuppression with SRL (trough levels 12–15 ng/mL for the first 3 months then

Table 2 Main characteristics of selected studies

Authors	Study design	Population, country	Sample size	Criteria for diagnosis of ovarian cyst	Ovaries study performed before treatment	Drug doses	Follow-up	Definition of clinically significant ovarian cyst
Cure *et al** updated by Del Olmo Garcia[12 13]	Cohort study	Women with T1DM who underwent AIT USA or multicentric	SRL+TAC: 13 mean age 41.0 (SD 8.8) years	Pelvic US (>3.0 cm in diameter that did not resolve spontaneously over 4 months)	See Olmo Garcia	TAC serum levels of 3–6 ng/mL SRL levels of 12–15 ng/mL for the first 90 days and 7–12 ng/mL thereafter.	24 months	>6 Four of the subjects (40%) underwent surgery
Gaber *et al*[14]	RCT	High-risk renal allograft recipients USA	SRL+TAC: 104 SRL+CsA: 98 Age not reported separately for women	Not reported	Not reported	SRL levels of 10–15 ng/mL TAC up to 0.2 mg/kg/day to achieve levels of 10–15 ng/mL between day 1 and week 2, 5–10 ng/mL between weeks 2 and 26, and 3–5 ng/mL between weeks 26 and 52	12 months	Not reported
Alfadhli *et al*[8]	Retrospective chart review	Women with T1DM who underwent AIT Canada	SRL+TAC: 57 women median age 42.5 44 (70.5%) premenopausal 13 (15.4%) post menopausal	Pelvic US (>2.5 cm in diameter)	Routine pretransplant abdominal ultrasound scans	SRL (trough levels 12–15 ng/mL for the first 3 months then 7–10 ng/mL thereafter) and TAC (target trough level 3–6 ng/ mL). TAC at higher doses (target trough levels 10 ng/mL) along with mycophenolate mofetil (1 g two times per day as tolerated)	Median 53.1 IQR 32.0–70.4 months	However, 14 subjects (42.4%) reported pelvic pain. In four cases, severe pelvic pain resulted in emergency room visits because of ovarian cyst rupture (n=2) or torsion (n=2).
Del Olmo Garcia *et al*[13]	Cohort study	Women with T1DM who underwent AIT USA or multicentric	SRL: 18 mean age 48.5 (SD 8.00) years	Pelvic US	Peritransplant ultrasound examination	SRL: serum levels 12–15 ng/mL for the first 90 days and 7–12 ng/mL thereafter	Mean 7.9 (SD 1.13) years	See cure

Continued

Table 2 Continued

Authors	Study design	Population, country	Sample size	Criteria for diagnosis of ovarian cyst	Ovaries study performed before treatment	Drug doses	Follow-up	Definition of clinically significant ovarian cyst
Braun *et al*[11]	RCT	Adult females with autosomal dominant polycystic kidney disease Switzerland	SRL: 21 (mean age 31) standard care=18 (mean age 32)	MRI without contrast material (>2 cm in diameter)	Abdominal MRI without contrast material	SRL 1.3–1.5 mg day	18 months	One patient presented with acute abdominal pain and a large cyst of the left ovary while receiving SRL and was cystectomised at 164 days after randomisation.
Ignjatović *et al*[15]	Retrospective chart review	Renal transplant recipients Serbia	SRL: 6 women converted from CNI Age not reported	Not reported	Basic physical examination	SRL: serum levels 7–10 ng/mL for months 6–12 after transplant, 5–10 ng/mL thereafter	Mean 65 (SD 20) months	Early after the conversion two of the patients developed serious crural oedema and multiple ovarian cysts with oligomenorrhea. After reconversion to CNI they lost oedema and ovarian cysts and returned to a regular period.
Bachmann *et al*[9]	Retrospective chart review	Renal transplant recipients Germany	mTORi: 102 other treatments: 469 (median age 32 for patients with OC)	Pelvic US	Ultrasound examination in the early postoperative period (<4 weeks)	SRL or everolimus (trough level 3–8 ng/mL)	41.9 months (range 4.5–307)	Surgery

*Excluded from the main analysis but included in the subanalysis for menopausal status (information not present in the updated report).
AIT, allogeneic islet transplantation; CNI, chronic calcineurin inhibitor; CsA, cyclosporine A; mTORi, mammalian target of rapamycin inhibitors; OC, ovarian cysts; RCT, randomised clinical trial; SRL, sirolimus; TAC, tacrolimus; T1DM, type 1 diabetes mellitus; US, ultrasound.

Table 3 Study quality evaluation according the Newcastle-Ottawa Scale (cohort studies and retrospective chart review)* or Cochrane risk of bias (randomised clinical trials)†

Publications							
Cohort studies and retrospective chart review		Selection		Comparability		Outcome	Study quality*
Cure *et al*[12]	1	*	1	*	1	*	6/9
	2	–	2	–	2	*	
	3	*			3	–	
	4	*					
Alfadhli *et al*[8]	1	*	1	*	1	*	7/9
	2	*	2	–	2	*	
	3	*			3	*	
	4	–					
Del Olmo Garcia *et al*[13]	1	*	1	–	1	*	5/9
	2	–	2	–	2	*	
	3	*			3	–	
	4	*					
Ignjatović *et al*[15]	1	*	1	–	1	–	5/9
	2	–	2	–	2	*	
	3	*			3	*	
	4	*					
Bachmann *et al*[9]	1	*	1	*	1	*	9/9
	2	*	2	*	2	*	
	3	*			3	*	
	4	*					
RCT							Overall risk of bias
Braun *et al*[10]	Randomisation: some concern						Low
	Assignment to intervention: low risk						
	Adhering to intervention: low risk						
	Missing outcome: low risk						
	Measure of outcome: low risk						
	Selection of results: low risk						
Gaber *et al*[14]	Randomization: low risk						Low
	Assignment to intervention: low risk						
	Adhering to intervention: low risk						
	Missing outcome: low risk						
	Measure of outcome: low risk						
	Selection of results: low risk						

A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

*We used the Newcastle-Ottawa quality assessment scale with maximum score 9, as presented at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed 24 April 2017). Most items were evaluated as '-' because of the small sample size or absence of not exposed cohort.

†For the assessment of randomised controlled studies, we used the revised Cochrane risk of bias tool as presented at https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2.

RCT, randomised controlled trial.

7–10 ng/mL thereafter) and TAC (target trough level 3–6 ng/ mL). A small group of patients received TAC at higher doses (target trough levels 10 ng/mL) along with mycophenolate mofetil (1 g two times per day as tolerated) for immunosuppression from the time of transplant. Ovarian cysts were found in 33 out of 57 women at

Table 4 Results of selected studies: patients with incident ovarian cyst on the total of treated women (%)

	SRL	SRL +TAC	SRL or everolimus	All	Standard treatment
Total series					
Gaber *et al*[14]	7/98* (7.14)	1/104 (0.96)		8/202 (7.84)	
Alfadhli *et al*[8]		33/57 (57.89)		33/57 (57.89)	
Del Olmo Garcia *et al*[13]	10/18 (55.56)			10/18 (55.56)	
Ignjatović *et al*[15]	2/6 (33.33)			2/6 (33.33)	
Comparative studies					
Braun *et al*[10]	12/21 (57.14)			12/21 (57.14)	5/18 (27.28)
Bachmann *et al*[9]			21/102 (20.59)	21/102 (20.59)	23/469 (4.26)
Total	*31/143 (21.68)*	*34/161 (21.12)*	*21/102 (20.59)*	*86/406 (21.18)*	*28/487 (5.75)*
Premenopause					
Cure *et al*[12]		7/9 (77.78)		7/9 (77.78)	
Alfadhli *et al*[8]		31/44 (70.45)		31/44 (70.45)	
Total		*38/53 (71.70)*		*38/53 (71.70)*	
Post menopause					
Cure *et al*[12]		1/4 (25.00)		1/4 (25.00)	
Alfadhli *et al*[8]		2/13 (15.38)		2/13 (15.38)	
Total		*3/17 (17.65)*		*3/17 (17.65)*	
Clinically significant					
Alfadhli *et al*[8]		14/57 (24.56)			
Del Olmo Garcia *et al*[13]	8/18 (44.44)				
Braun *et al*[10]	1/21 (4.76)				0/18 (0.00)
Bachmann *et al*[9]			10/102 (9.80)		8/487
Total	*9/39 (23.08)*	*14/57* (24.56)	*10/102* (9.80)		*8/505 (1.58)*

*Sirolimus (SRL)+cyclosporine.
TAC, tacrolimus.

a median of 235 (119–405) days after the first islet transplantation: 31 out of 44 (70.5%) premenopausal and 2 out of 13 (15.4%) postmenopausal women (p=0.001). Ovarian cysts occurred more frequently in subjects taking SRL plus TAC than those taking high doses of TAC plus mycophenolate mofetil (33/53, 62.3%, vs 0/4, 0%, p=0.027). No women using combined oral contraception developed ovarian cysts. Among women taking SRL, average SRL trough levels were similar between those who developed ovarian cysts and those who did not (median 12.1, IQR 10.9–13.3, vs 12.2, IQR 11.5–12.6 ng/mL, p=0.993).

SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects. The median maximal cyst diameter was 6.0 (3.8–7.6) cm. Most cysts were asymptomatic and noted incidentally on routine imaging. However, 14 subjects (42.4%) reported pelvic pain. In four cases, severe pelvic pain resulted in emergency room visits because of ovarian cyst rupture (n=2) or torsion (n=2). Histology was benign in all cases.

Del Olmo Garcia *et al*[13] reported a total of 18 subjects (mean age at transplantation 48.5, SD 8.0 years) with T1DM, who underwent allogeneic transplantation and were treated with SRL, given orally pretransplant, at 0.2 mg/kg, and then adjusted to achieve trough levels of 12–15 ng/mL for the first 90 days and 7–12 ng/mL thereafter. After the transplant, they were followed for a mean time of 7.9 (SD 1.13) years. In this study, a total of ten ovarian cysts (56%) were observed. All the cysts were benign, but eight were considered complex because of haemorrhage, hydrosalpinx, cyst's size (>6 cm), spontaneous rupture or need of surgery for resolution. Four women (40%) underwent cystectomy because of poor response to medical treatment. Part of this sample (13 out of 18) was previously described by Cure *et al*,[12] who reported that in four women, postmenopausal at the time they were transplanted, one case of ovarian cyst was observed, whereas out of nine premenopausal women seven developed ovarian cysts.

Braun *et al*[10] reviewed the occurrence of ovarian cysts in a post hoc analysis of an open label randomised controlled phase II trial, conducted between March 2006 and March 2010. Women with autosomal dominant polycystic kidney disease were treated with 1.3–1.5 mg SRL per day for a

median of 19 months (N=21) or standard care (N=18). Ovarian cysts were observed in 12 out of 21 patients in the SRL group, compared with 5 out of 18 patients in the control group (HR 4.4, 95% CI 1.1 to 26). Differences in ovarian cysts between SRL and control did not seem to depend on the contraceptive method (barrier methods: 7 out of 11 and 3 out of 9 patients in the SRL and control groups; oral contraceptives: 5 out of 10 and 2 out of 9 patients in the SRL and control groups). Clinical significance of ovarian cysts was not reported. One patient presented with acute abdominal pain and a large cyst of the left ovary while receiving SRL and underwent surgery.

Ignjatović *et al*[15] reviewed 24 transplant patients (6 women) who switched from calcineurin inhibitors (CNI) to SRL from 2003 to 2011. Patients converted from CNI to SRL, with target serum levels 7–10 ng/mL for months 6–12 after transplant, and 5–10 ng/mL thereafter. Early after the conversion, two patients developed ovarian cysts with oligomenorrhea and reconverted to CNI, with cyst resolution and return to regular period.

Bachmann *et al*[9] compared the effect of mTORi versus non-mTORi immunosuppression on the incidence, size and complication rate of ovarian cysts in renal transplant recipients. They retrospectively analysed 571 consecutive female kidney transplant patients between 2000 and 2008; they were followed-up till December 2012. Of those, 102 (17.8%) patients received mTORi for at least 1 month after transplantation. A total of 44 women (7.7%) with new ovarian cysts were reported, 21 among patients receiving mTORi (20.5%) and 23 in the control group (4.9%). This difference was statistically significant (p<0.001). The hospitalisation rate was also more frequent in the mTOR group, with 21 hospitalisations in 10 mTORi patients versus 9 hospitalisations in 8 control subjects (p=0.05). Ten women in the mTORi group (9.8%) versus eight in the control group (1.7%) had symptomatic, clinically significant ovarian cysts requiring surgery.

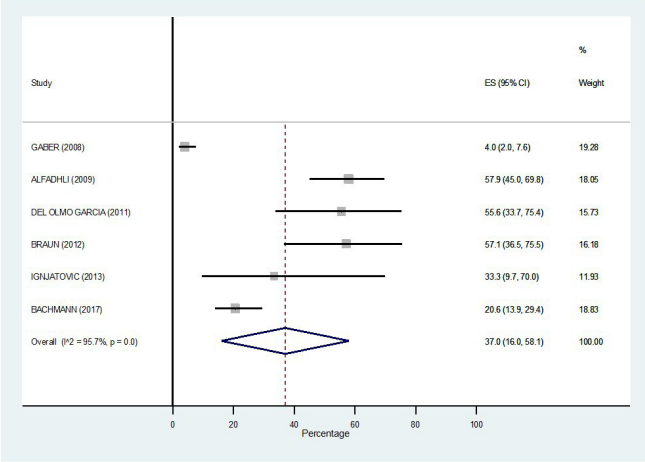

Figure 2 Forest plot of ovarian cyst incidence. ES, estimate.

Synthesis of results

Overall, 406 women were treated with mTORi in the studies included in this meta-analysis and 86 developed ovarian cysts. The frequency of ovarian cysts in women treated with mTORi, without any specific restriction regarding the type of drug, is reported in table 5.

As shown in table 5, the pooled incidence was 37.0% (95% CI 16.0% to 58.1%) (figure 2). The rate of ovarian cysts ranged from 4.0% to 57.9%, leading to a remarkable heterogeneity (χ^2 for heterogeneity 115.0, p<0.001, I^2=95.7%). Excluding the study with most extreme results[14] the pooled estimate increased to 44.9% (95% CI 23.7% to 66.1%), with a small decrease of heterogeneity, that remained, however, remarkable.

As shown in table 4, pooling the results of two studies,[8 12] we found that ovarian cyst rates were higher for premenopausal women (38/53, 71.7%) than postmenopausal ones (3/17, 17.6%) and the difference was statistically significant (p<0.0001): the OR for developing ovarian cysts was 12.46 (95% CI 3.04 to 50.98) comparing premenopausal with postmenopausal women.

Table 5 Pooled estimates of ovarian cyst incidence

Authors	Cases	Sample size	Pooled incidence estimate	95% CI
Gaber *et al*[14]	8	202	4.0	2.0 to 7.6
Alfadhli *et al*[8]	33	57	57.9	45.0 to 69.8
Del Olmo Garcia et al[13]	10	18	55.6	33.7 to 75.4
Braun *et al*[10]	12	21	57.1	36.5 to 75.5
Ignjatovic et al[15]	2	6	33.3	9.7 to 70.0
Bachmann *et al*[9]	21	102	20.6	13.9 to 29.4
Random pooled estimate			37.0	16.0 to 58.1
Heterogeneity χ^2=115.0 (d.f.=5) p=0.0; I^2 (variation in estimate attributable to heterogeneity)=95.7% Estimate of between-study variance τ^2=0.1				
Random pooled estimate excluding Gaber *et al*			44.9	23.7 to 66.1
Heterogeneity χ^2=32.5 (d.f.=4) p=0.0; I^2 (variation in estimate attributable to heterogeneity)=87.7% Estimate of between-study variance τ^2=0.1				

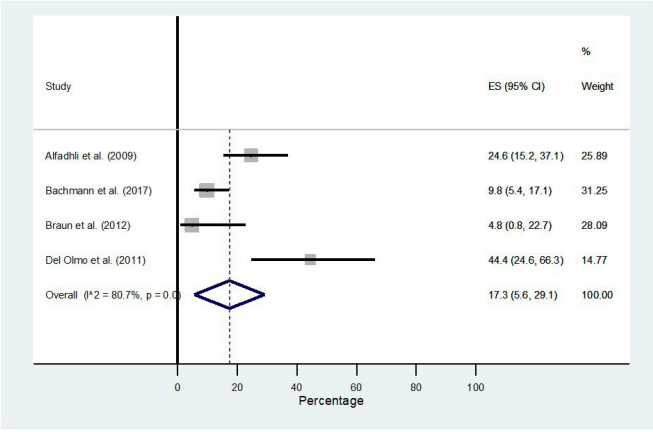

Figure 3 Forest plot of clinically significant ovarian cyst incidence. ES, estimate.

Two studies compared mTORi versus non-mTORi immunosuppression.[9 10] The pooled OR for ovarian cyst incidence was 4.62 (95% CI 2.58 to 8.28) and the pooled OR for clinically significant ovarian cysts was 5.56 (95% CI 2.34 to 14.67).

Finally, we pooled the incidence of clinically significant ovarian cysts in studies reporting this information.[8–10 12] The resulting estimate was 17.3% (95% CI 5.6% to 29.1%), heterogeneity χ^2 15.5, p<0.001) (figure 3).

DISCUSSION

This systematic review shows that, in women treated with mTORi, the incidences of ovarian cysts ranged between less than 10% to more than 50%, in different studies. The pooled incidence was 37%, 17% only considering clinically significant ovarian cysts. The risk seems to be higher among premenopausal women: two studies distinguished ovarian cyst incidence occurring in premenopausal and postmenopausal patients, with consistent results,[8 12] suggesting that mTORi effect is higher in presence of spontaneous ovarian activity.

Where immunosuppression was achieved using mTORi as compared with non-mTORi,[9 10] women on mTORi were at higher risk of developing ovarian cysts.

The limited data and the differences in the presentation of results do not provide the opportunity of analysing in detail the role of stopping mTORi on the clinical course of ovarian cysts or the protective role of oral contraceptive use.

In the study by Alfadhli *et al*,[8] SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects; however, the proportion with partial or complete cyst resolution was similar in those who did or did not discontinue SRL (12/15, 80%, vs 6/8, 75%, p=NS).

Another potential risk factor for the development of ovarian cysts during mTORi use was a previous history of ovarian cysts; Del Olmo Garcia *et al*[13] reported five patients with such a history.

In our synthesis, we found an extremely high heterogeneity, that may be due to both the different criteria for diagnosis of ovarian cysts and the type of disease requiring mTORi use. For example, T1DM is associated with menstrual irregularity and PCOS,[17 18] hence a higher basal frequency of ovarian cyst development. Another factor likely affecting heterogeneity was the active screening for ovarian cysts development in women on mTORi treatment. In particular, it appears that in the study with the lowest incidence,[14] women did not routinely undergo abdominal scans: pelvic (ie, transvaginal) sonography would be the preferred imaging modality to exclude ovarian cysts.

In biological terms, mTORi may affect the levels of LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone). Further, expression of progesterone receptors can be inhibited by SRL via the mTOR and inhibition of progesterone receptors in the ovaries may interfere with ovarian cysts development. However, the specific mechanisms linking mTORi exposure and risk of developing ovarian cysts are unknown.[12]

This review and meta-analysis may be affected by potential limitations or bias.

Findings from this systematic review and meta-analysis are based on an extremely limited number of studies; thus the results should be considered cautiously. Taking this aspect into account, the general results confirm clinical suggestions that mTORi increase the frequency of benign ovarian cysts.

Among studies, the heterogeneity was remarkable. This finding may be due to the characteristics of the selected samples. First, two studies included women with T1DM who underwent AIT,[8 13] three included women who underwent renal transplantation[9 14 15] and one study women with autosomal dominant polycystic kidney.[10] Then, the ascertainment of ovarian cysts was performed with different methodologies, variable imaging modalities and definitions (size, persistence) of ovarian cyst. Whereas certain studies defined ovarian cysts as cystic formation >2 cm in MRI images,[11] other studies included only cysts of >3 cm not resolving spontaneously after 4 months diagnosed by transvaginal sonography[12 13] and in two studies the method was not reported.[14 15] Thus, in order to reduce the heterogeneity in the definition of ovarian cysts among the considered studies, we have also performed a meta-analysis of clinically significant ovarian cysts. Lastly, the number of study participants was quite different among studies ranging between 6 and more than 200 women. Despite this, the pooled estimate is not overwhelmingly affected by the largest studies,[9 14] and the study weights are similar (figure 2).

We considered only publications published in English. Authors may be more prone to publish in an international, English-language journal if results are positive, whereas negative findings are more often published in local journals.[19] Limiting our analysis to publications in English language journals can therefore restrict the completeness of information, thereby causing bias. The

direction and the strength of this bias are not however clear.

Another limitation is the fact that most of studies included an extremely limited number of subjects. Although systematic reviews with meta-analyses provide an explicit method for synthesising evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study. Lastly, this study was not registered a priori.

Despite these limitations, consistent results among all studies give strong support to the general findings.

Although the biological and clinical explanation of the results of our analysis is not totally clear, observational studies and clinical trials consistently suggest that ovarian cysts are a common adverse effect of mTORi. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery. Based on these considerations, women and physicians should be warned in routine clinical practice about the gynaecological impact of long-term use of mTORi. Further the risk of ovarian cyst, together with the impact of mTORi on glucose metabolism, risk of diabetes and other potential adverse effects should be included in the risk benefit balance of mTORi use as immunosuppressive agents.

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REFERENCES

1 Zheng Y, Jiang Y. mTOR inhibitors at a glance. *Mol Cell Pharmacol* 2015;7:15–20.
2 Alam H, Maizels ET, Park Y, *et al*. Follicle-stimulating hormone activation of hypoxia-inducible factor-1 by the phosphatidylinositol 3-kinase/AKT/Ras homolog enriched in brain (Rheb)/mammalian target of rapamycin (mTOR) pathway is necessary for induction of select protein markers of follicular differentiation. *J Biol Chem* 2004;279:19431–40.
3 Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6:e1000097.
4 Wells G, Shea B, O'Connell D, *et al*. The Newcastle-Ottawa scale (NOS) for assessing the quality of non randomised studies in meta-analyses n.d. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
5 Sterne JAC, Savović J, Page MJ, *et al*. Rob 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019;366:1–8.
6 Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health* 2014;72:39.
7 Greenland S. Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* 1987;9:1–30.
8 Alfadhli E, Koh A, Albaker W, *et al*. High prevalence of ovarian cysts in premenopausal women receiving sirolimus and tacrolimus after clinical islet transplantation. *Transpl Int* 2009;22:622–5.
9 Bachmann F, Glander P, Budde K, *et al*. High incidence of ovarian cysts in women receiving mTOR inhibitors after renal transplantation. *J Womens Health* 2018;27:394–8.
10 Braun M, Young J, Reiner CS, *et al*. Low-Dose oral sirolimus and the risk of menstrual-cycle disturbances and ovarian cysts: analysis of the randomized controlled SUISSE ADPKD trial. *PLoS One* 2012;7:e45868.
11 Braun M, Young J, Reiner CS, *et al*. Ovarian toxicity from sirolimus. *N Engl J Med* 2012;366:1062–4.
12 Cure P, Pileggi A, Froud T, *et al*. Alterations of the female reproductive system in recipients of islet grafts. *Transplantation* 2004;78:1576–81.
13 Del Olmo Garcia MI, Lauriola V, Aracena AG, *et al*. Alterations of the female reproductive system in islet recipient receiving immunosuppression. *Cell Transplant* 2011;20:1649–51.
14 Gaber AO, Kahan BD, Van Buren C, *et al*. Comparison of sirolimus plus tacrolimus versus sirolimus plus cyclosporine in high-risk renal allograft recipients: results from an open-label, randomized trial. *Transplantation* 2008;86:1187–95.
15 Ignjatović L, Hrvacević R, Jovanović D, *et al*. Conversion from calcineurin inhibitors to sirolimus of recipients with chronic kidney graft disease grade III for a period 2003-2011. *Vojnosanit Pregl* 2013;70:848–53.
16 Ryan EA, Paty BW, Senior PA, *et al*. Five-Year follow-up after clinical islet transplantation. *Diabetes* 2005;54:2060–9.
17 Strotmeyer ES, Steenkiste AR, Foley TP, *et al*. Menstrual cycle differences between women with type 1 diabetes and women without diabetes. *Diabetes Care* 2003;26:1016–21.
18 Escobar-Morreale HF, Roldán B, Barrio R, *et al*. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2000;85:4182–7.
19 Grégoire G, Derderian F, Le Lorier J. Selecting the language of the publications included in a meta-analysis: is there a tower of Babel bias? *J Clin Epidemiol* 1995;48:159–63.